ELSEVIER

Schizophrenia Research 47 (2001) 49–58

SCHIZOPHRENIA RESEARCH

www.elsevier.com/locate/schres

Lack of association between the T→C 267 serotonin 5-HT_6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia

Mario Masellis [a], Vincenzo S. Basile [a], Herbert Y. Meltzer [b], Jeffrey A. Lieberman [c], Serge Sevy [d], David A. Goldman [e], Mark W. Hamblin [f], Fabio M. Macciardi [a], James L. Kennedy [a,*]

[a] *Neurogenetics Section, Clarke Division, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ont. M5T 1R8, Canada*

[b] *Department of Psychiatry, Vanderbilt University, Nashville, TN, USA*

[c] *Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA*

[d] *Department of Veteran Affairs, The Bronx Veteran's Administrative Medical Center, New York, NY, USA*

[e] *Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD, USA*

[f] *GRECC, VA Puget Sound Health Care System, and Department of Psychiatry and Behavioural Sciences, University of Washington, Seattle WA, USA*

Received 13 July 1999; accepted 7 January 2000

Abstract

The affinity of clozapine for 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7, and 5-HT_{1A} receptors has been suggested to contribute to various aspects of its complex clinical actions. This study examined the hypothesis that genetic variation in 5-HT_{1A}, 5-HT_6, and 5-HT_7 receptor genes is involved in the variability observed in response to clozapine. We employed a pharmacogenetic approach in a group ($n=185$) of schizophrenia patients that have been clinically well characterized for clozapine response. Polymorphisms in the 5-HT_6 (HTR6), 5-HT_{1A} (HTR1A) and 5-HT_7 (HTR7) receptor genes were genotyped. No evidence for either an allelic or genotypic association of the T→C 267 HTR6 polymorphism with response to clozapine was found in our sample (allele: $\chi^2=0.06$, 1 df, $P=0.80$; genotype: $\chi^2=1.21$, 2 df, $P=0.55$). The pro16leu HTR1A polymorphism was not observed in our sample; all individuals genotyped were pro/pro 16 homozygotes. With respect to the pro279leu HTR7 polymorphism, one Caucasian male responder to clozapine was observed to be heterozygous (pro/leu 279 genotype). This individual was clinically similar to the other clozapine responders. Overall, our findings do not support a role for the T→C 267 polymorphism of the 5-HT_6 receptor gene in response to clozapine, although replication is required to confirm this finding.

Keywords: Clozapine response; Pharmacogenetic; Polymorphism; Serotonin receptor

* Corresponding author. Tel.: +1-416-979-4987; fax: +1-416-979-4666.
E-mail address: james_kennedy@camh.net (J.L. Kennedy)

0920-9964/01/$ - see front matter
PII: S0920-9964(00)00016-5

1. Introduction

Response to the atypical antipsychotic, clozapine, is highly variable among individuals who have treatment-refractory and/or intolerant schizophrenia (Bleehen, 1993). The ability to predict response to clozapine would be important from a clinical perspective: predicted responders to this drug could be treated preferentially, whereas those less likely to respond could be offered other possible treatments. However, finding specific and consistent predictors of response to clozapine has proven to be a difficult task [reviewed by Meltzer (1996)]. Clozapine's wide spectrum of unique clinical effects for the treatment of schizophrenia [reviewed by Meltzer (1995)] suggests that many factors, both biological and environmental, are involved in the phenotype of response.

Pharmacogenetics seeks to identify genetic variation (polymorphism) in or near the coding region of genes that encode protein structures involved in the action of a drug, and correlate these with variability in the clinical profile, e.g. its pattern of response and/or side effects. This paradigm can be applied to predict the variable responsiveness to clozapine. Recent advances in molecular genetics allow for the identification of genes involved in complex traits (Bennett et al., 1995). These techniques are well suited for examining the trait of clozapine response, as it is likely complex and multifactorial in nature.

Clozapine has a high affinity for many receptors from multiple neurotransmitter systems. All of these pharmacological interactions have been implicated in its mechanism of action [reviewed by Ashby and Wang (1996)]. In particular, clozapine has moderate to high affinity for at least five subtypes of serotonin receptors (Meltzer, 1994; Meltzer, 1999). The current study examined polymorphisms in the following candidate receptor genes from the serotonin system: 5-HT_{1A} (HTR1A), 5-HT_6 (HTR6), and 5-HT_7 (HTR7). There are several lines of evidence implicating a role for these serotonin receptor proteins in the mechanism of action of clozapine.

Newman-Tancredi et al. (1996) have demonstrated that clozapine acts as a partial agonist at 5-HT_{1A} receptors. The effect of clozapine to increase extracellular dopamine levels in rat prefrontal cortex is believed to be important in its ability to improve positive and negative symptoms (Kuroki et al. 1998). This effect is blocked by pretreatment with the 5-HT_{1A} antagonist, WAY 100635 (Rollema et al., 1997; Ichikawa and Meltzer, unpublished data), suggesting it is mediated by the partial agonist effect of clozapine. The combination of a 5-HT_{1A} agonist, 8-OH-DPAT, with haloperidol produces a clozapine-like pattern of activation of the early intermediate gene, c-fos, in rat prefrontal cortex, nucleus accumbens, and striatum (Tremblay et al., 1998). The ability of clozapine to decrease serotonin efflux in the rat ventral hippocampus also is partially dependent upon its 5-HT_{1A} agonist properties (Bengtsson et al., 1998). Thus, there is significant preclinical evidence to suggest 5-HT_{1A} agonism is a meaningful component of the action of clozapine.

5-HT_{1A} agonism has also been shown to prevent and ameliorate neuroleptic-induced catalepsy in rodents (Broekkamp et al., 1988; Invernizzi et al., 1988; Hicks, 1990; Wadenberg and Ahlenius, 1991; Wadenberg, 1992; Neal-Beliveau et al., 1993). This effect has also been observed in primate models of extrapyramidal symptoms (EPS) (Liebman et al., 1989; Casey, 1992, 1994). For a review, refer to Kapur and Remington (1996).

From a neuroanatomical perspective, 5-HT_{1A} receptor sites are localized in many areas of the brain, including the hippocampus, the septum, the amygdaloid, the neocortex, the hypothalamus, and the raphe nuclei, particularly the dorsal raphe [reviewed by Hoyer et al. (1994)]. Many of these areas have been implicated as neuroanatomical substrates of schizophrenia. Based on this information, the 5-HT_{1A} receptor gene (HTR1A) is an important candidate to test with respect to clozapine response.

HTR1A is located on human chromosome 5q and a cytosine to thymidine (C→T) polymorphism in this gene, which causes an amino acid substitution of proline to leucine at position 16 of the 5-HT_{1A} receptor protein (pro16leu), has been identified in a Japanese sample (Inayama et al., 1995). This polymorphism was genotyped in our group of clozapine-treated patients.

The human 5-HT_6 receptor gene (HTR6) has

been cloned and localized to chromosome 1p (Kohen et al., 1996). A silent thymidine to cytosine polymorphism at position 267 (T→C 267), within the first exon of HTR6, has been identified (Kohen et al., 1996). Given that a weak positive association has been reported between this candidate receptor gene variant and response to clozapine in a Chinese sample (Yu et al., 1999), we have evaluated this polymorphism in our sample of clozapine-treated patients.

mRNA for the 5-HT_6 receptor has been found in several human brain regions, including the caudate nucleus, the hippocampus, and the amygdala; lower levels were observed in the thalamus, subthalamic nuclei, and the substantia nigra (Kohen et al., 1996). Several of these brain areas have been implicated in the pathophysiology of schizophrenia. Clozapine and several other atypical antipsychotic agents are antagonists at, and have demonstrated high affinity for, 5-HT_6 receptors (Roth et al., 1994; Glatt et al., 1995; Kohen et al., 1996). Approximately, 40% of clozapine binding sites in the rat brain pharmacologically resemble the 5-HT_6 receptor (Glatt et al., 1995), and based on relative affinities of clozapine for D2 and 5-HT_{2A} receptors, 5-HT_6 receptors should be highly occupied at clinically relevant doses of clozapine (Kohen et al., 1996). This suggests that 5-HT_6 receptors may be important in the mechanism of action of clozapine and other atypical antipsychotic agents.

Clozapine is an antagonist at, and possesses high affinity for, 5-HT_7 receptors (Roth et al., 1994). mRNA and in situ hybridization studies have demonstrated that 5-HT_7 receptors may be expressed in the brain in the hypothalamus, the anteroventral and paraventricular thalamic nuclei, and the hippocampus [reviewed by Eglen et al. (1997)]. Expression of mRNA in these midline, thalamic, and limbic structures suggests a role of 5-HT_7 in the regulation of emotion (Eglen et al., 1997) and some of these structures may be involved in the pathophysiology of schizophrenia. There is some evidence which suggests that the 5-HT_7 receptor subtype may be involved in the regulation of circadian rhythm phase shifts (Lovenberg et al., 1993; Ying and Rusak, 1997). This role of the 5-HT_7 receptor may be interesting, considering that some people afflicted with schizophrenia experience a reversal of the sleep–wake cycle accompanied by severe insomnia (Benca, 1996).

The gene encoding the 5-HT_7 receptor has been localized to human chromosome 10q (Gelernter et al., 1995). A cytosine to thymidine (C→T) polymorphism, leading to a proline to leucine amino acid substitution at position 279 (pro279leu) in the third intracellular loop of the receptor protein, was genotyped in our group of clozapine-treated patients (Erdmann et al., 1996; Pesonen et al., 1998).

2. Methods

2.1. Clinical sample

Clinical data from patients with DSM-III-R diagnoses of schizophrenia (treatment-refractory or intolerant; Kane et al., 1988) were obtained from: Case Western Reserve University in Cleveland (HY Meltzer, $n=105$); Hillside Hospital in Long Island (JA Lieberman, $n=65$); and the Bronx Veteran's Administrative Medical Center in New York (S Sevy, $n=16$). Treatment response was evaluated at 6 months or more using the following criteria: (1) $\geq$20% reduction in Brief Psychiatric Rating Scale (BPRS) total score, or (2) if a patient seemed clinically improved and demonstrated a $\geq$15% but $<$20% decrease in BPRS, a reduction of at least one category on the Clinical Global Impressions (CGI) scale may have been considered to augment response definition; eight of the 185 patients were classified as responders using this additional criterion. Sample characterization has been described in detail elsewhere (Masellis et al., 1998).

2.2. Laboratory methods

Blood samples were collected from the clinical sites and sent to the Clarke Division, Centre for Addiction and Mental Health in Toronto. Genomic DNA was extracted from white blood cells using the high-salt method (Lahiri and Nurnberger, 1991). All genotyping of the patients' DNA was performed blind to the psychiatric

ratings. Biallelic polymorphisms in the three candidate genes, HTR1A, HTR6, and HTR7, were genotyped in our group of clozapine-treated patients. The laboratory techniques for each of these will now be described.

2.2.1. HTR1A

The pro16leu polymorphism, resulting from a C→T 47 polymorphism in the HTR1A locus, was genotyped employing allele-specific oligonucleotide (ASO) hybridization. The polymerase chain reaction (PCR) was used to amplify a 411 base pair region of HTR1A from position −66 to 345. The forward and reverse PCR primers used were 5′-aag ggg cga ggc gaa tct tcg cgc tg-3′, and 5′-gag ggc gat gaa cag gtc gca ggt tac-3′, respectively. Primer sequences were obtained from Inayama et al. (1995). The PCR reaction was performed using 150 ng of genomic DNA, 1.0 mM $MgCl_2$, 0.6 μM of both forward and reverse primer, 160 μM of each nucleotide, and one unit of AmpliTaq DNA polymerase (Perkin–Elmer) in a final reaction volume of 25 μl. The PCR program consisted of 30 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s using a GeneAmp 9600 Perkin–Elmer Cetus PCR machine. After PCR amplification, 5 μl of the amplification product was diluted in 200 μl of 15 × SSC, which was then denatured into single-stranded DNA by boiling followed by snap-cooling. This was then applied onto Hybond-N+ membrane by slot-blotting, dividing the product equally between wells in two lanes. The end result was two sets of identical blots. The blots were then UV-crosslinked and were prehybridized in 5 × SSPE, 6% PEG, 5 × Denhardt's solution and 0.5% SDS for 1 h at 35°C. ASOs were designed with the following sequences pro16 ASO: 5′-cac cac *c* ggc tcc-3′, and leu16 ASO: 5′-cac cac *t* ggc tcc-3′. These ASOs were end-labelled with $\gamma^{32}P$, and the pro16 ASO was hybridized to one set of the identical blots, while the leu16 ASO was hybridized to the other (hybridization temperature: 35°C; hybridization time: 1 h). The blots were washed in 3 × SSC/0.1% SDS at 44°C for the leu16 allele, and 46°C for the pro16 allele to remove the non-complementary oligonucleotide. The blots were then subjected to autoradiography to visualize the variants.

2.2.2. HTR6

The T→C 267 polymorphism at the HTR6 locus was genotyped employing the PCR and restriction fragment length polymorphism (PCR-RFLP) technique. A modified protocol of Kohen et al. (1996) was used. Primer sequences were as follows: forward, 5′-tgc tga tcg cgc tca tct gca ctc a-3′; reverse, 5′-ctg cag cgt ctc cga ggc ctg act g-3′. The PCR reaction was performed using 200 ng of genomic DNA, 1.0 mM $MgCl_2$, 0.8 μM of both forward and reverse primer, 200 μM each of dATP, dCTP, 90% dGTP + 10% deaza-dGTP, and dTTP, 10% dimethyl sulfoxide (DMSO), and one unit of AmpliTaq DNA polymerase (Perkin–Elmer) in a final reaction volume of 25 μl. The PCR program consisted of 45 cycles of 95°C for 40 s, 60°C for 40 s, and 72°C for 40 s using a GeneAmp 9600 Perkin–Elmer Cetus PCR machine. The PCR products were then restriction digested using RsaI following the manufacturer's protocol (New England Biolabs). The digested products were electrophoresed on 3.5% agarose gels, which were stained with ethidium bromide for UV visualization. RsaI recognizes a restriction site created by the C267 variant producing two fragments of length 449 bp and 129 bp, but leaves the T267 variant uncut (578 bp).

2.2.3. HTR7

The pro279leu polymorphism at the HTR7 locus was genotyped employing the PCR-RFLP technique. Pesonen et al. (1995, 1998) first described this polymorphism, and we obtained the specific methodological details from D. Goldman. Forward and reverse primer sequences were as follows: 5′-gat tct ctc cgt ctg gct tct-3′ and 5′-gca cac tct tcc acc tcc ttc-3′, respectively. The PCR reaction was performed using 150 ng of genomic DNA, 1.0 mM $MgCl_2$, 0.6 μM of both forward and reverse primer, 160 μM of each nucleotide, and one unit of AmpliTaq DNA polymerase (Perkin–Elmer) in a final reaction volume of 25 μl. The PCR program consisted of 30 cycles of 95°C for 30 s, 61°C for 30 s, and 72°C for 30 s using a GeneAmp 9600 Perkin–Elmer Cetus PCR machine. The PCR products were then restriction digested using XhoI following the manufacturer's protocol (New England Biolabs). The digested

products were electrophoresed on 3.0% agarose gels, which were stained with ethidium bromide for UV visualization. XhoI recognizes the restriction site responsible for the pro279 variant producing two fragments of length 230 bp and 72 bp, but leaves the leu279 variant uncut (302 bp).

2.3. Statistical methods

The categorical data were analyzed using χ^2 tests, and the continuous data, e.g. age, were analyzed using analysis of variance (ANOVA). The statistical program used was the Statistical Package for the Social Sciences (SPSS), version 8.0. Power analysis was performed using Epi Info, Version 5.01a (Public Domain Software for Epidemiology and Disease Surveillance, March 1991).

3. Results

There were no statistically significant differences in ethnicity and response rate between the patients from the three clinical sites. There was a significant difference observed between the mean age of the patients from the three clinical sites [$F(2, 181) = 3.43$, $P = 0.007$]; using Tukey's HSD test for post hoc comparisons, the mean ages of the Meltzer and Lieberman samples were significantly lower than that of the Sevy sample (SS). There was also a significant difference in the proportion of males to females between the SS and the two larger samples (HYM and JAL). The smaller sample consisted predominantly of males ($\chi^2 = 8.09$, 2 df, $P = 0.02$, two-tailed). The samples were combined into one group and a total of 185 patients was evaluated: 132 men, 53 women; 144 Caucasians, 40 African-Americans, one Asian; the mean age was 33.7 years (SD 8.9). Of these, 97 (52.4%) patients were considered responders to clozapine by the criteria cited above, and 88 (47.6%) were not. In this combined sample, age, sex and ethnicity factors were not different between responders and non-responders to clozapine. Refer to Masellis et al. (1998) for additional demographic details.

Genotype frequencies for the T→C 267 HTR6 polymorphism were not significantly different between patients from the different centres, between patients from different ethnic backgrounds, or between males and females. With respect to clozapine response, there were no significant differences in either the HTR6 allele or genotype counts [$\chi^2 = 0.06$, 1 df, $P = 0.80$ (two-tailed); $\chi^2 = 1.21$, 2 df, $P = 0.55$ (two-tailed) respectively] between responders and non-responders (see Table 1). There was no deviation from Hardy–Weinberg equilibrium for the HTR6 polymorphism in either the responders or non-responders to clozapine. Our allele frequencies were also comparable to those published by Kohen et al. (1996).

The HTR1A pro16leu amino acid substitution was not found to be polymorphic in our sample; all individuals were pro/pro 16 homozygotes. The leu279 variant of the pro279leu HTR7 polymorphism was observed in only one heterozygous individual (pro/leu 279 genotype). This patient was a 23 year old Caucasian male and was a responder to clozapine. There were no outstanding clinical features that distinguished this individual from the rest of the responders.

4. Discussion

We have found no evidence of an association between the T→C 267 polymorphism in the

Table 1
Allele and genotype counts and frequencies of the T→C 267 polymorphism in the 5-HT_6 receptor gene (HTR6) in clozapine-treated patients

	Responders	Non-responders	Total
Allele[a]			
T267	26 (14%)	25 (15%)	51 (15%)
C267	156 (86%)	139 (85%)	295 (85%)
Total	182	164	346
Genotype[b]			
T/T 267	4 (4%)	2 (2%)	6 (3%)
T/C 267	18 (20%)	21 (26%)	39 (23%)
C/C 267	69 (76%)	59 (72%)	128 (74%)
Total	91	82	173

[a] $\chi^2 = 0.06$, 1 df, $P = 0.80$ (two-tailed) for clinical response relative to allele counts (odds ratio for T267 association to non-response: 1.08; 95% CI 0.57–2.05).

[b] $\chi^2 = 1.21$, 2 df, $P = 0.55$ (two-tailed) for clinical response relative to genotype counts.

5-HT_6 receptor gene and clinical response to clozapine in our sample of schizophrenia patients. This is contrary to the findings of Yu et al. (1999) who observed that patients with the homozygous genotype T/T 267 were more likely to be responders to clozapine than those with either the C/T 267 or C/C 267 genotypes (total $n=99$). This discrepancy may be due to methodological differences between the studies. In particular, Yu et al. (1999) used ANOVA to assess for differences in raw BPRS score changes, after clozapine treatment, among genotypic groups for the HTR6 polymorphism; our study employed the non-parametric χ^2 statistic to compare differences in the allelic and genotypic counts between responders and non-responders to clozapine. This may account for the observed discrepancy between our study and that of Yu et al. (1999). However, we had complete BPRS data for the patients ascertained at the Case Western Reserve University site ($n=105$; contributor HYM), and this allowed us to perform a similar analysis (analysis of covariance: ANCOVA), in this sample, to that employed by Yu et al. (1999). After baseline BPRS scores were controlled for by incorporating them into the ANCOVA model as covariates, there were no statistically significant differences observed among means for post-clozapine BPRS score changes for each HTR6 genotypic group [$F(2, 82)=2.01$, $P=0.14$, $n=86$; results not shown]. Therefore, it is unlikely that this methodological difference accounted for the discrepancy noted between our study and that of Yu et al. (1999), although our power was low for this type of analysis ($1-\beta=41\%$). For a thorough discussion of the ANOVA/ANCOVA approach to genetic analyses, refer to Basile et al. (1999).

The T→C 267 HTR6 polymorphism does not alter the predicted amino acid sequence of the receptor. However, an unidentified functional HTR6 polymorphism, which confers response/non-response to clozapine, may be in linkage disequilibrium with this T→C 267 HTR6 variant in the Chinese sample of Yu et al. (1999), but not in our sample. This may also account for the discrepancy between our study and that of Yu et al. (1999). Alternatively, their finding may be a false positive result (Yu et al., 1999). Another explanation may be that the ethnic heterogeneity of our North American sample may have produced a type II error. However, we attempted to control for this by ensuring that our responder and non-responder groups were as similar as possible with respect to ethnic composition. Overall, our result does not support a role for the T→C 267 polymorphism of the 5-HT_6 receptor gene in the variable phenotype of clozapine response. It would be important to identify other polymorphisms in HTR6, as these may be involved in predicting clozapine response.

Power analysis for this HTR6 polymorphism revealed that our sample has the ability to detect an odds ratio as low as 2.33 for a T267 allelic association with non-response, specifying a type I error rate $\alpha=0.05$, power $(1-\beta)=0.80$, and the proportion of responders with the T267 risk allele for non-response $P_0=0.11$. Therefore, our sample has the required power to detect an association, even if the T→C 267 HTR6 polymorphism provided only a minor contribution to the overall variability observed in response to clozapine.

Several polymorphisms have been identified at the HTR1A locus (Khan et al., 1990; Melmer et al., 1991; Warren et al., 1992; Bolos et al., 1993; Brett et al., 1995; Erdmann et al., 1995; Inayama et al., 1995; Nakhai et al., 1995; Xie et al., 1995; Lam et al., 1996). Based on the low genetic information of these polymorphisms, we chose to examine the pro16leu HTR1A polymorphism identified by Inayama et al. (1995), which demonstrated a high degree of genetic informativeness in a Japanese sample.

The pro16leu HTR1A polymorphism was initially identified and genotyped in a sample of unrelated Japanese bipolar disorder patients and healthy controls using single-stranded conformational polymorphism (SSCP) analysis (Inayama et al., 1995). We have employed an ASO hybridization technique to examine this polymorphism in our sample of clozapine-treated patients. In our sample, all individuals genotyped were homozygous for the pro16 variant. Inayama et al. (1995) found frequencies for the rarer leu16 variant of 2% and 31% in bipolar patients and controls, respectively. However, when testing their data for Hardy–Weinberg equilibrium between the alleles

of this HTR1A polymorphism, we found that the genotype frequencies of their control population deviated significantly from the frequencies expected under random mating conditions.

SSCP is a valid technique to screen for mutations; however, its sensitivity to external conditions, and its lack of specificity when genotyping a single polymorphism in a large sample of individuals, increases the likelihood of making spurious genotyping errors. The observed deviation from Hardy–Weinberg equilibrium in the control sample of Inayama et al. (1995) may be accounted for by the use of the SSCP methodology.

This pro16leu HTR1A polymorphism may only exist in Asian populations, and thus we were unable to detect it in our North American sample. This polymorphism may be involved in clozapine response in the Asian population but not in other ethnic groups; therefore, future studies of this HTR1A polymorphism and response to clozapine in Asian samples may be warranted. Overall, given the low genetic informativeness of the polymorphisms identified in HTR1A, to date, it would be appropriate to conduct a haplotype analysis using several markers in the vicinity of this gene as this may increase the power of the analysis.

The pro279leu variant at the HTR7 locus was observed in only one individual in our sample, who had no distinguishing clinical characteristics. This polymorphism is located within the third intracellular cytoplasmic loop of the 5-HT7 receptor protein and the substitution of pro279 to leu279, based on secondary structure analysis, is postulated to change local protein structure and thus affect coupling to G-proteins (Pesonen et al., 1998). The rare occurrence of the leu279 variant, and the rarity of other known HTR7 variants (Gelernter et al., 1995; Erdmann et al., 1996; Pesonen et al., 1998), suggests that, even if functionality of this polymorphism is confirmed, it would only be playing a role in a small number of patients. It would be important to identify other, more common, polymorphisms in HTR7 to further assess its role in the observed variability in response to clozapine.

In terms of pharmacogenetics of clozapine response and serotonin genes, in general, previous studies of clozapine response and the serotonin system have examined the 5-HT_{2A} and 5-HT_{2C} receptor genes (Arranz et al., 1995, 1996, 1998; Masellis et al., 1995, 1998; Nothen et al., 1995; Sodhi et al., 1995; Malhotra et al., 1996a,b; Rietschel et al., 1997; Lin et al., 1999). These studies have yielded largely inconsistent results. Apart from the obvious reasons of type I and type II errors, these inconsistencies may also be due to the methodological differences among the studies [Masellis et al., 1998; reviewed in Masellis et al. (2000)]. Recently, several recommendations have been made that attempt to address these methodological differences among pharmacogenetic studies of clozapine response and for discussion; refer to Rietschel et al. (1999) and Masellis et al. (2000). It is also possible that different genes are operating in various clinical groups of patients, making it difficult to attain consistent findings. In other words, variability in one particular candidate gene contributes to the phenotype of response in a particular group of patients, whereas that in different candidate genes may be operating in others (i.e., genetic heterogeneity). Alternatively, polymorphisms across several candidate genes may interact, and thus each may contribute a small proportion to the total variance observed in the phenotype of clozapine response (i.e., polygenic inheritance). These concepts are plausible given the genetic heterogeneity of schizophrenia, the involvement of multiple loci in the disorder (McGuffin et al., 1995), in addition to the complex mechanism of action of clozapine. Our current study has focused on variation within the serotonin receptor genes, HTR1A, HTR6, and HTR7, and has extended our previous work examining the putative relationship between receptor gene polymorphisms and clozapine response.

It may be important to consider other factors in clozapine response, including environmental influences and genetic variability in clozapine metabolism (Ozdemir et al., 1999). In the future, the employment of molecular genetic association approaches to pharmacogenetics may allow for the analysis of candidate genes across several biological systems, each contributing to only a small amount of the total variance observed in the trait of clozapine response.

Acknowledgements

MM was supported by a Department of Psychiatry, Faculty of Medicine, University of Toronto Summer Scholarship. This work was supported by a Medical Research Council of Canada (MT15007) grant and a NARSAD Independent Investigator Award to JLK.

References

Arranz, M., Collier, D., Sodhi, M., Ball, D., Roberts, G., Price, J., Sham, P., Kerwin, R., 1995. Association between clozapine response and allelic variation in 5-HT2A receptor gene. Lancet 346, 281–282.

Arranz, M.J., Collier, D.A., Munro, J., Sham, P., Kirov, G., Sodhi, M., Roberts, G., Price, J., Kerwin, R.W., 1996. Analysis of a structural polymorphism in the 5-HT2A receptor and clinical response to clozapine. Neuroscience Letters 217, 177–178.

Arranz, M.J., Munro, J., Owen, M.J., Spurlock, G., Sham, P.C., Zhao, J., Kirov, G., Collier, D.A., Kerwin, R.W., 1998. Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. Molecular Psychiatry 3 (1), 61–66.

Ashby, C.R., Wang, R.Y., 1996. Pharmacological actions of the atypical antipsychotic drug clozapine: a review. Synapse 24 (4), 349–394.

Basile, V.S., Masellis, M., Badri, F., Paterson, A.D., Meltzer, H.Y., Lieberman, J.A., Potkin, S.G., Macciardi, F., Kennedy, J.L., 1999. Association of the MscI polymorphism of the dopamine D3 receptor gene with tardive dyskinesia in schizophrenia. Neuropsychopharmacology 21 (1), 17–27.

Benca, R.M., 1996. Sleep in psychiatric disorders. Neurologic Clinics 14 (4), 739–764.

Bengtsson, H.J., Kullberg, A., Millan, M.J., Hjorth, S., 1998. The role of 5-HT1a autoreceptors and alpha 1-adrenoceptors in the modulation of 5-HT release — III. Clozapine and the novel putative antipsychotic S 16924. Neuropharmacology 37, 349–356.

Bennett, S.T., Lucassen, A.M., Gough, S.C., Powell, E.E., Undlien, D.E., Pritchard, L.E., Merriman, M.E., Kawaguchi, Y., Dronsfield, M.J., Pociot, F., et al., 1995. Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. Nature Genetics 9 (3), 284–292.

Bleehen, T., 1993. Clozapine: Literature Review. Sandoz Pharma Ltd, Basle.

Bolos, A.M., Goldman, D., Dean, M., 1993. Dinucleotide repeat and alu repeat polymorphisms at the 5-HT1A (HTR1A) receptor gene. Psychiatric Genetics 3, 235–240.

Brett, P.M., Curtis, D., Robertson, M.M., Gurling, H.M., 1995. Exclusion of the 5-HT1A serotonin neuroreceptor and tryptophan oxygenase genes in a large British kindred multiply affected with Tourette's syndrome, chronic motor tics and obsessive–compulsive behavior. American Journal of Psychiatry 152 (3), 437–440.

Broekkamp, C.L., Oosterloo, S.K., Berendsen, H.H., van Delft, A.M., 1988. Effect of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. Naunyn–Schmiedebergs Archives of Pharmacology 338 (2), 191–195.

Casey, D.E., 1992. The effect of 8-OH DPAT on haloperidol-induced dystonia in non-human primates Paper presented at. 31st Annual Meeting of the American College of Neuropsychopharmacology, San Juan, Puerto Rico.

Casey, D.E., 1994. Extrapyramidal syndromes: primate models as predictors of clinical response Paper presented at. 33rd Annual Meeting of the American College of Neuropsychopharmacology, San Juan, Puerto Rico.

Eglen, R.M., Jasper, J.R., Chang, D.J., Martin, G.R., 1997. The 5-HT7 receptor: orphan found. Trends in Pharmacological Sciences 18 (4), 104–107.

Erdmann, J., Shimron-Abarbanell, D., Cichon, S., Albus, M., Maier, W., Lichtermann, D., Minges, J., Reuner, U., Franzek, E., Ertl, M.A., et al., 1995. Systematic screening for mutations in the promoter and the coding region of the 5-HT1A gene. American Journal of Medical Genetics 60 (5), 393–399.

Erdmann, J., Nothen, M.M., Shimron-Abarbanell, D., Rietschel, M., Albus, M., Borrmann, M., Maier, W., Franzek, E., Korner, J., Weigelt, B., Fimmers, R., Propping, P., 1996. The human serotonin 7 (5-HT7) receptor gene: genomic organization and systematic mutation screening in schizophrenia and bipolar affective disorder. Molecular Psychiatry 1, 392–397.

Gelernter, J., Rao, P.A., Pauls, D.L., Hamblin, M.W., Sibley, D.R., Kidd, K.K., 1995. Assignment of the 5HT7 receptor gene (HTR7) to chromosome 10q and exclusion of genetic linkage with Tourette syndrome. Genomics 26 (2), 207–209.

Glatt, C.E., Snowman, A.M., Sibley, D.R., Snyder, S.H., 1995. Clozapine: selective labeling of sites resembling 5HT6 serotonin receptors may reflect psychoactive profile. Molecular Medicine 1 (4), 398–406.

Hicks, P.B., 1990. The effect of serotonergic agents on haloperidol-induced catalepsy. Life Sciences 47 (18), 1609–1615.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacological Reviews 46 (2), 157–203.

Inayama, Y., Yoneda, H., Inada, Y., Sakai, J., Koh, J., Hayashi, K., Kono, Y., Nonomura, Y., Asaba, H., Sakai, T., 1995. HTR1A pro16/leu16 polymorphism in bipolar affective disorder Paper presented at World Congress on Psychiatric Genetics, Cardiff, UK.

Invernizzi, R.W., Cervo, L., Samanin, R., 1988. 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a selective serotonin1A receptor agonist, blocks haloperidol-induced catalepsy by an

action on raphe nuclei medianus and dorsalis. Neuropharmacology 27 (5), 515–518.

Kane, J., Honigfeld, G., Singer, J., Meltzer, H., 1988. Clozapine for the treatment-resistant schizophrenic: a double-blind comparison with chlorpromazine. Archives of General Psychiatry 45, 789–796.

Kapur, S., Remington, G., 1996. Serotonin–dopamine interaction and its relevance to schizophrenia. American Journal of Psychiatry 153, 466–476.

Khan, M.A., Isenberg, K.E., Aschauer, H., Devor, E.J., 1990. A Sac I RFLP is detected with the 5-HT1a serotonin receptor probe G21. Nucleic Acids Research 18 (3), 691

Kohen, R., Metcalf, M.A., Khan, N., Druck, T., Huebner, K., Lachowicz, J.E., Meltzer, H.Y., Sibley, D.R., Roth, B.L., Hamblin, M.W., 1996. Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. Journal of Neurochemistry 66 (1), 47–56.

Kuroki, T., Meltzer, H.Y., Ichikawa, J., 1998. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucelus accumbens. Journal of Pharmacology and Experimental Therapeutics 288, 774–781.

Lahiri, D.K., Nurnberger Jr, J.I., 1991. A rapid non-enzymatic method for the preparation of HMV DNA from blood for RFLP studies. Nucleic Acids Research 19 (19), 5444

Lam, S., Shen, Y., Nguyen, T., Messier, T.L., Brann, M., Comings, D., George, S.R., O'Dowd, B.F., 1996. A serotonin receptor gene (5HT1A) variant found in a Tourette's syndrome patient. Biochemical and Biophysical Research Communications 219 (3), 853–858.

Liebman, J.M., Gerhardt, S.C., Gerber, R., 1989. Effects of 5-HT1A agonists and 5-HT2 antagonists on haloperidol-induced dyskinesias in squirrel monkeys: no evidence for reciprocal 5-HT–dopamine interaction. Psychopharmacology 97 (4), 456–461.

Lin, C.H., Tsai, S.J., Yu, Y.W., Song, H.L., Tu, P.C., Sim, C.B., Hsu, C.P., Yang, K.H., Hong, C.J., 1999. No evidence for association of serotonin-2A receptor variant (102 T/C) with schizophrenia or clozapine response in a Chinese population. Neuro. Report 10, 57–60.

Lovenberg, T.W., Baron, B.M., de Lecea, L., Miller, J.D., Prosser, R.A., Rea, M.A., Foye, P.E., Racke, M., Slone, A.L., Siegel, B.W., Danielson, P.E., Sutcliffe, J.G., Erlander, M.G., 1993. A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythyms. Neuron 11, 449–458.

Malhotra, A.K., Goldman, D., Ozaki, N., Breier, A., Buchanan, R., Pickar, D., 1996a. Lack of associaton between polymorphisms in the 5-HT2A receptor gene and the antipsychotic response to clozapine. American Journal of Psychiatry 153 (8), 1092–1094.

Malhotra, A.K., Goldman, D., Ozaki, N., Rooney, W., Clifton, A., Buchanan, R.W., Breier, A., Pickar, D., 1996b. Clozapine response and the 5HT(2C)Cys(23)Ser polymorphism. Neuroreport 7 (13), 2100–2102.

Masellis, M., Paterson, A.D., Badri, F., Lieberman, J.A., Meltzer, H.Y., Cavazzoni, P., Kennedy, J.L., 1995. Genetic variation of 5-HT2A receptor and response to clozapine. Lancet 346, 1108

Masellis, M., Basile, V., Meltzer, H.Y., Lieberman, J.A., Sevy, S., Macciardi, F.M., Cola, P., Howard, A., Badri, F., Nothen, M.M., Kalow, W., Kennedy, J.L., 1998. Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. Neuropsychopharmacology 19 (2), 123–132.

Masellis, M., Basile, V.S., Ozdemir, V., Meltzer, H.Y., Macciardi, F.M., Kennedy, J.L., 2000. Pharmacogenetics of antipsychotic treatment: lessons learned from clozapine. Biological Psychiatry 47, 252–266.

McGuffin, P., Owen, M.J., Farmer, A.E., 1995. Genetic basis of schizophrenia. Lancet 346 (8976), 678–682.

Melmer, G., Sherrington, R., Mankoo, B., Kalsi, G., Curtis, D., Gurling, H.M., 1991. A cosmid clone for the 5HT1A receptor (HTR1A) reveals a TaqI RFLP that shows tight linkage to dna loci D5S6, D5S39 and D5S76. Genomics 11 (3), 767–769.

Meltzer, H.Y., 1994. An overview of the mechanism of action of clozapine. Journal of Clinical Psychiatry 55, suppl. B, 47–52.

Meltzer, H.Y., 1995. Role of serotonin in the action of atypical antipsychotic drugs. Clinical Neuroscience 3, 64–75.

Meltzer, H.Y., 1996. Predictors of response to clozapine. Psychiatric Annals 26 (7), 385–389.

Meltzer, H.Y., 1999. The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 21, 2S, 106–115.

Nakhai, B., Nielsen, D.A., noila, M., Goldman, D., 1995. Two naturally occurring amino acid substitutions in the human 5-HT1A receptor: glycine 22 to serine 22 and isoleucine 28 to valine 28. Biochemical and Biophysical Research Communications 210 (2), 530–536.

Neal-Beliveau, B.S., Joyce, J.N., Lucki, I., 1993. Serotonergic involvement in haloperidol-induced catalepsy. Journal of Pharmacology and Experimental Therapeutics 265 (1), 207–217.

Newman-Tancredi, A., Chaput, C., Verriele, L., Millan, M.J., 1996. Clozapine is a partial agonist at cloned, human serotonin 5-HT1A receptors. Neuropharmacology 35 (1), 119–121.

Nothen, M.M., Rietschel, M., Erdmann, J., Oberlander, H., Moller, H.J., Naber, D., Propping, P., 1995. Genetic variation of the 5-HT2A receptor and response to clozapine. Lancet 346, 908–909.

Ozdemir, V., Masellis, M., Basile, V.S., Kalow, W., Meltzer, H.Y., Lieberman, J.A., Kennedy, J.L., 1999. Variability in response to clozapine: potential role of cytochrome P450 1A2 and the dopamine D4 receptor gene. CNS Spectrums 4 (6), 30–56.

Pesonen, U., Koulu, M., Eggert, M., Virkunnen, M., Linnoila, M., Goldman, D., 1995. Rare non-conservative amino acid substitution in the 5-HT7 receptor gene found in alcoholics with antisocial personality disorder Paper presented at. 25th Annual Meeting of the Society for Neuroscience, San Diego.

Pesonen, U., Koulu, M., Bergen, A., Eggert, M., Naukkarinen, H., Virkkunen, M., Linnoila, M., Goldman, D., 1998.

Mutation screening of the 5-hydroxytryptamine7 receptor gene among Finnish alcoholics and controls. Psychiatry Research 77 (3), 139–145.

Rietschel, M., Naber, D., Fimmers, R., Moeller, H.J., Propping, P., Noethen, M.M., 1997. Efficacy and side-effects of clozapine not associated with variation in the 5-HT2C receptor. Neuroreport 8, 1999–2003.

Rietschel, M., Kennedy, J.L., Macciardi, F., Meltzer, H.Y., The Consensus Group for Outcome Measures in Psychoses for Pharmacological Studies 1999. Application of Pharmacogenetics to Psychotic Disorders: the First Consensus Conference, 12 July 1998, Glasgow. Schizophrenia Research 37 (2), 191–196.

Rollema, H., Lu, Y., Schmidt, A.W., Zorn, S.H., 1997. Clozapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. European Journal of Pharmacology 338, R3–R5.

Roth, B.L., Craigo, S.C., Choudhary, M.S., Uluer, A., Monsma Jr, F.J., Shen, Y., Meltzer, H.Y., Sibley, D.R., 1994. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. Journal of Pharmacology and Experimental Therapeutics 268 (3), 1403–1410.

Sodhi, M.S., Arranz, M.J., Curtis, D., Ball, D.M., Sham, P., Roberts, G.W., Price, J., Collier, D.A., Kerwin, R.W., 1995. Association between clozapine response and allelic variation in the 5-HT2C receptor gene. Neuroreport 7 (1), 169–172.

Tremblay, P.O., Gervai, S.J., Rouillard, C., 1998. Modification of haloperidol-induced pattern of c-fos expression by serotonin. European Journal of Neuroscience 1, 3546–3555.

Wadenberg, M.L., 1992. Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. Journal of Neural Transmission General Section 89, 49–59.

Wadenberg, M.L., Ahlenius, S., 1991. Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. Journal of Neural Transmission General Section 83 (12), 43–53.

Warren Jr, J.T., Peacock, M.L., Fink, J.K., 1992. An RsaI polymorphism in the human serotonin receptor gene (HTR1A): detection by DGGE and RFLP analysis. Human Molecular Genetics 1 (9), 778

Xie, D.W., Deng, Z.L., Ishigaki, T., Nakamura, Y., Suzuki, Y., Miyasato, K., Ohara, K., Ohara, K., 1995. The gene encoding the 5-HT1A receptor is intact in mood disorders. Neuropsychopharmacology 12 (3), 263–268.

Ying, S.W., Rusak, B., 1997. 5-HT7 receptors mediate serotonergic effects on light-sensitive suprachiasmatic nucleus neurons. Brain Research 755 (2), 246–254.

Yu, Y.W., Tsai, S.J., Lin, C.H., Hsu, C.P., Yang, K.H., Hong, C.J., 1999. Serotonin-6 receptor variant (C267T) and clinical response to clozapine. Neuroreport 10 (6), 1231–1233.